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Short Communication

Preliminary Qualitative Phytochemical Screening of Edible Mushroom *Hypsizygus ulmarius*

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Abstract

Mushrooms are white rot fungi regarded as one of the well known food and possessing various kinds of biopharmaceuticals compounds. The present study aimed for screening and determining the bioactive compounds present in edible mushroom *Hypsizygus ulmarius*. *Hypsizygus ulmarius* was analyzed for total ash, water soluble extractive value, alcohol soluble extractive value and moisture content and which was found to be 4.31, 2.8, 0.4 and 10.4% /5gm of the sample powder respectively with light flourescene characters. Qualitative phytochemical screening showed the presence of alkaloids, phenolics, saponins, tannins, glycosides, carbohydrates and proteins. Total phenolic and flavonoid content of *Hypsizygus ulmarius* was found to be 30.5mg pyrocatechol and 32.24mg Quercetin equivalent /g of dry mushroom powder respectively. *Hypsizygus ulmarius* may be considered as good source phytochemicals that can be used in pharmaceutical, medical and food additives (antioxidant). Furthermore, screening and characterization of secondary metabolites is warranted.

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INTRODUCTION

Edible mushrooms are nutritionally endowed fungi (mostly Basidiomycetes) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Chang and Miles, 1992; Lindequist *et al.,* 2005). They are documented as being rich in proteins, minerals, vitamins while they are low in lipids (Pathak *et al.,* 1997). Mushrooms have long been appreciated for their excellent sensory characteristics, flavor and texture. They are now recognized as a nutritious food as well as an important valuable source of biologically active compounds (Rajewska *et al.,* 2004). Mushrooms comprise an untapped source of powerful new pharmaceutical products (Rai mehandra *et al.,* 2005). Nearly 10,000 mushroom species are known, of which 2000 are safe for humans and about 300 of them possess medicinal properties. In the last decades, the wide range of pharmaceutically interesting metabolites from basidiomycetes, a large group of terrestrial fungi of the phylum Basdiomycota, has been one of the most attractive groups of natural products studied (Boh *et al.*, 2003). Mushrooms are the source of unlimited polysaccharides with antitumor and immune-stimulating properties (Jose *et al.,* 2000).

The use of mushrooms with potential therapeutic properties raises global interest from the scientific and clinical community based on two main reasons. First, mushrooms demonstrate their efficiency against numerous diseases and metabolic disturbances (Poucheret *et al.,* 2006). Second, the medicinal use of edible mushrooms extracts seems to be a more natural, less expensive approach and in general involves minimal unwanted side effects (Poucheret *et al.,* 2006). Moreover, purified bioactive compounds derived from edible mushrooms might be a potentially important new source of therapeutic agents. Documented literature indicates that mushrooms have phytochemicals and other compounds which are strong antioxidants (Fang *et al.,* 2002; Liu *et al.,* 2004). In the present investigation edible mushroom *Hypsizygus ulmarius* is studied for its preliminary photochemical. *Hypsizygus ulmarius* is commonly called as elm oyster which is a high yielding edible mushroom for which commercial cultivation technology has been released and is gaining popularity. Previous reports suggests that the mushroom is rich in antioxidants and proved for its antidiabetic activity (Meera *et al.,* 2011). The presence of laccase enzyme, their purification and characterization was reported by Ravikumar *et al.,* 2012. The effect of laccase from *Hypsizygus ulmarius* in decolorization of different dyes is reported (Ravikumar *et al.,* 2013). The present study is designed to screen and to determine the bioactive compounds present in edible mushroom *Hypsizygus ulmarius*.

MATERIAL AND METHODS

All the chemicals and solvents used were of analytical grade and were obtained from Sigma Chemical Co. (St. Louis, MO) and Hi Media chemicals, Mumbai. The mushroom *Hypsizygus ulmarius* (DMRP-253) were grown in association with vinayaka mushroom cultivators, Hebbel, Bangalore, Karnataka.

Sample Preparation

Fresh mushroom sample(1kg) were randomly selected, air dried in shade and powdered. About 50gms of the powdered mushroom was extracted with 400ml of methanol (40-50°C), using soxhlet apparatus (9-cycles) and filtered through muslin cloth. The filtered extract was evaporated under reduced pressure and vacuum dried to get viscous residue. The sample obtained is used to study physical properties and for further estimations of phytochemical analysis (Shirmila *et al.,* 2013).

Physiochemical Studies

The dried mushroom powder was subjected to physicochemical evaluation such as total ash, alcohol soluble extractive value, water soluble extractive value and total moisture content according to standard method (Indian Pharmacopoeia Commission, 2007).

Fluorescence Characters

Fluorescence characters of powdered *Hypsizygus ulmarius* mushroom were studied both in day light and UV light according to the method described by Shanti *et al.,* 2012; Kulkarni *et al.,* 2011.

Preliminary (Qualitative) Phytochemical Screening

The preliminary phytochemical analysis were carried out according to the method of Kokate *et al.,* 1994; Khandelwal *et al.,* 2000. The extracts obtained were subjected to various chemical tests to detect the chemical constituents present in them.

Determination of Total Phenolics

Total soluble phenol in the methanol extracts was determined with Folin–Ciocalteu reagent, according to the method of Slinkard *et al.,* 1977 using pyrocatechol as a standard. Briefly, 1 ml of extract solution in a volumetric flask was diluted glass-distilled water (46ml). Folin–Ciocalteu reagent (1 ml) was added and the contents of the flask were mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The concentration of total phenol compounds in the mushroom methonol extract, determined as micrograms of pyrocatechol equivalents, obtained from the standard pyrocatechol graph.

Determination of Total Flavonoids

The total flavonoid content was measured according to the method of (Atanassova *et al.,* 2011,) with an $AlCl_3$ colorimetric assay. Aliquots of 1 ml of fractions extracts and standard solution of Quercetin (100µg/ml) were transferred in to 10 ml volumetric flasks containing 4 ml of distilled water. 0.3 ml of 5% NaNO2 was added in each flask followed by 0.3% of AlCl3. At the sixth minute, 2 ml of 1 M NaOH was added and the absorbance was measured against a prepared reagent blank at 410 nm with an UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). All Samples were analyzed in duplicates.

RESULT AND DISCUSSION

New scientific strategies are required for the evaluation of natural products with specific biological activities which requires large screening process. The physical properties of methanolic extract (Table 1) showed the following characters.

The Physicochemical studies like moisture content; extractive values and ash content (Table 2) are used to determine quality and purity (Mahendra *et al.,* 2009; Shirmila *et al.,* 2013). Shade Dried *Hypsizygus ulmarius* mushroom contain high moisture content about 10.4% quite similar to *Ganoderma lucidum* (10.54%, w/w) High moisture content may lead to the activation of enzymes and promotes susceptibility to microbial growth, which accelerates spoilage (Usman *et al.,* 2012). Variation in water content among the mushroom samples could be caused by the nature of the mushrooms and the different environmental growth factors such as temperature and relative humidity of the metabolic water. The elimination of water content of the sample to dry state will increase the concentration of nutrient relatively. Thus, drying mushrooms is one method that would extend the shelf life of mushrooms by reducing unnecessary biochemical reaction such as enzymatic browning and lipid oxidation that may lead to quality deterioration (Mattila *et al.,* 2002). High ash content of a drug gives an idea about earthy matter or inorganic composition and other impurities present along with the drug. The results of the present studies show that the ash content values (4.31%) are lesser than those reported for *Ganoderma lucidum(*5.93%, w/w) (Usman *et al.,* 2012). Extractive values give an indication about the nature of the chemical constituents present in the drug. Alcohol soluble extractive(2.8%) were higher as compared to Water soluble extractives (0.4%), which showed that *Hypsizygus ulmarius* had more alcohol soluble polar constituents. Each determination was carried out three times and the average value was taken.

Table 1: Properties and percentage yield of the extracts obtained from dry *Hypsizygus ulmarius* powder.

Properties of the methanolic extract	
Weight of mushroom powder used for extraction	50g
Methanolic extract procured	9.76g (19.52%)
Color	Brownish
Consistency	Semisolid and sticky

Table 2: Physciochemical parameters of *Hypsizygus ulmarius* mushroom powder.

No	Parameters	% w/w
1	Total ash content	4.31
2	Water soluble extractive value	0.4
	Alcohol soluble extractive value	2.8
3	Moisture content	10.4

Fluorescence analysis of dried *Hypsizygus ulmarius* mushroom powder showed various colors in visible, when compared to short and long UV (Table 3). Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range but many natural products do not visibly flourescent in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Kamilmulla *et al.,* 2010).

The qualitative preliminary phytochemical analysis of methanolic extract of *Hypsizygus ulmarius* revealed the presence of alkaloids, saponins, phenolic compounds, proteins, carbohydrates tannins and glycosides as reported by Egwim *et al.,* 2011; kadiri *et al.,* 1992 (Table 4). The presence of alkaloids in the mushroom powder

Table 3: Fluorescent analysis of *Hypsizygus ulmarius* dried powder.

No	Sample + Reagents	Short 254nm	Long 366nm	Visible Light
1	Drug powder	Cream	Cream	Cream
2	Powder + aqueous sodium hydroxide	Cream	Cream	Brown
3	Powder + alkaline sodium hydroxide	Cream	Cream	Light Brown
4	Powder + 1Nhydrochloric acid	Cream	Cream	Light Cream
5	Powder + 50% sulphuric acid	Green	Cream	Light Brown
6	Powder + 50% nitric acid	Cream	Light Brown	Light Cream
7	Powder + picric acid	Yellow	Cream	Yellow
8	Powder + acetic acid	Yellow	Cream	Light Brown
9	Powder + ferric chloride	Green	Brown	Light Brown
10	Powder + nitric acid + ammonia	Cream	Brown	Cream

explains its anti-bacterial activity. It is known that saponins inhibit Na^+ efflux by blockage of the influx of concentration in the cells, activating $Na^+ – Ca^{2+}$ antiporter in cardiac muscles. The increase in Ca^{2+} influx through this antiporter strengthens the contraction of heart muscles (Schneider *et al.,* 2004).

Table 4: Phytochemical screening of methonolic extract of *Hypsizygus ulmarius*.

Phytochemical	Tests	Result
Alkaloids	Mayer's	+++
	Wagner's	+++
	Dragendorff's	+++
	Hager's	+
Saponin	Froth's	++
Phenolics	Ferric chloride	+++
	Lead acetate	+
	Alkaline reagent	+++
	Shinoda's	+
Proteins	Millons	+
	Biuret	+++
	Ninhydrin	+++
Carbohydrates	Molisch's	+
	Benedict's	+
	Fehling's	+
Tannins	Vanillin hydrochloric	+++
	Gelatin	–
Glycosides	Legal's	++
	Borntrager's	–

+++: Highly present, ++: Moderately present, +: Faintly present, –: Absent

The total phenolic content of *Hypsizygus ulmarius* estimated by folin ciocalteau method were found to be 30.5mg/g catechol equivalent which is lower when compared to *pleurotus florida* (62.82mg) (Menaga *et al.,* 2012) and higher than agaricus species (Barros *et al.,* 2008) and Portuguese wild edible mushroom (Barros *et al.,* 2007) per gm of gallic acid equivalent. Phenolic compounds are antioxidants, and exhibit a wide range of spectrum of medicinal properties such as anti cancer, anti inflammatory and diabetic effects (Hamzah *et al.,* 2013).

The total flavonoids content estimated by aluminium chloride technique in terms of quercetin were found to be 32.24mg/gm of dry *Hypsizygus ulmarius* mushroom powder which is higher than that found in and edible mushroom in recent study Hamzah *et al.,* 2014). Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. Flavonoids are one of the most diverse group of natural compounds that have been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, antiallergenic, antiviral, anti inflammatory and vasodilating actions (Parajuli *et al.,* 2012; Pereira *et al.,* 2009). Thus the extracts of the studied mushrooms may be good alternatives for the treatment of diseases associated.

CONCLUSION

The results of the study reveals that, the crude methanolic extract of *Hypsizygus ulmarius* showed the presence of alkaloids, saponins, phenolic compounds, proteins, carbohydrates tannins and glycosides. The phenolics and flavonoids present could be an accessible source of natural antioxidant and antibiotics. Furthermore, isolation and characterization of pharmacologically active metabolites from *Hypsizygus ulmarius* is under process.

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